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Activity of the Triazole SCH 56592 against Disseminated Murine Coccidioidomycosis

JON E. LUTZ,[1,2,3] KARL V. CLEMONS,[1,2,3]* BEATRIZ H. ARISTIZABAL,[2] AND DAVID A. STEVENS[1,2,3]

Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center,[1] and California Institute for Medical Research,[2] San Jose, California 95128, and Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University, Stanford, California 94305[3]

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SCH 56592 (SCH) is a new triazole antifungal with a broad spectrum of activity. In vitro susceptibility testing against five strains of *Coccidioides immitis* revealed MICs from 0.39 to 3.13 μg/ml and minimal fungicidal concentrations from 1.56 to 3.13 μg/ml. A murine model of systemic coccidioidomycosis was established in female CD-1 mice. Groups received either no treatment or oral therapy with fluconazole at 10 or 100 mg/kg of body weight; itraconazole at 10 or 100 mg/kg; SCH at 0.5, 2, 10, or 25 mg/kg; or its methylcellulose diluent alone. Therapy began 2 days postinfection and continued once daily for 19 days. Surviving mice were euthanized 49 days postinfection, and infectious burdens were determined by culture. All drugs were superior to no-treatment or diluent-treatment controls ($P < 0.001$) in prolonging survival but were not significantly different from one another. Itraconazole at 100 mg/kg was superior to fluconazole in reduction of CFU in the spleen, liver, and lung ($P < 0.01$ to 0.001). SCH at 0.5 mg/kg was superior to either fluconazole or itraconazole at 10 mg/kg in reduction of CFU in all three organs ($P < 0.05$ to 0.001). SCH at 2 mg/kg was not significantly different from itraconazole at 100 mg/kg in all three organs. SCH at 10 and 25 mg/kg was superior to either dose of fluconazole or itraconazole in all three organs ($P < 0.05$ to 0.001). In terms of reduction of CFU, SCH was ≥200-fold as potent as fluconazole and ≥50-fold as potent as itraconazole. There was a clear dose-responsive relationship for SCH in each of the organs. It is noteworthy that SCH effected cures (no detectable *C. immitis* in any organ) in 1 of 9, 6 of 10, or 9 of 9 surviving mice in animals given 2, 10, or 25 mg/kg, respectively. Neither fluconazole nor itraconazole cured any survivor. SCH has potent, fungicidal activity in vivo against *C. immitis*. It should be considered for clinical trials in patients with coccidioidomycosis.

The dimorphic fungus *Coccidioides immitis* is endemic to the southwestern United States, as well as parts of Central and South America. It is estimated that there are at least 100,000 new infections annually in the United States (19). In a recent multiyear epidemic in California, the incidence increased 10-fold over baseline (2, 19). Compromised hosts, such as persons with human immunodeficiency virus infection, are especially susceptible to disseminated infection and death (11). Standard therapy for disseminated disease or chronic pulmonary disease has been either amphotericin B or one of several currently available azoles, e.g., miconazole, ketoconazole, fluconazole, or itraconazole (19). Amphotericin B and miconazole require intravenous administration (intrathecal or intraventricular in the case of meningitis) and are plagued by bothersome side effects (19). The currently available oral azoles represent a therapeutic advance. However, failures and relapses occur, particularly in meningitis (9, 10, 19). Thus, there is a definite need for improved antifungal drugs with favorable side effect and pharmacokinetic profiles for the treatment of coccidioidomycosis.

The new antifungal triazole, SCH 56592 (Fig. 1), has been studied in a variety of in vitro and in vivo fungal models. It has activity in vitro against several *Candida* species superior to that of itraconazole and fluconazole against all isolates tested (16). Activity has been demonstrated in vitro against all four serotypes of *Cryptococcus neoformans*, with MICs lower than those of amphotericin B and fluconazole for all isolates tested (18). It also is active in vitro against *Aspergillus* spp. (16), as well as in murine models of both pulmonary and systemic aspergillosis, in which it was more potent than itraconazole administered at 100 mg/kg of body weight. Activity against systemic infections caused by *Candida albicans* has been shown in both immunocompetent and gamma-irradiated mice, as well as in a hamster model of vaginitis (1, 17). Against the yeast phase of *Blastomyces dermatitidis*, SCH 56592 was more active in vitro than either amphotericin B, itraconazole, or fluconazole (20). Also, when tested against *B. dermatitidis* in vivo, all doses of SCH 56592 prolonged the survival of mice and resulted in the sterilization of lungs in the highest dosage group, similar to that achieved with amphotericin B, whereas itraconazole at much higher doses failed to sterilize the lungs (20). SCH 56592 has favorable pharmacokinetics, with bioavailability of orally administered drug ranging from 12% in cynomolgus monkeys to 49% in rats (15). The half-life is 18 h in dogs and 22 h in cynomolgus monkeys (15), suggesting that once-daily dosing is possible.

In this report, we describe the in vitro activity of SCH 56592 against several isolates of *C. immitis* and the results of a murine model of systemic infection with *C. immitis*, which show the promising activity of SCH 56592 against *C. immitis*.

MATERIALS AND METHODS

In vitro assays. The in vitro activity of SCH 56592 was tested against five isolates of *C. immitis*; these included the Silveira strain and four other clinical isolates. Susceptibility testing was done for each isolate in 3-ml broth dilution assays in a synthetic medium, Synthetic Amino Acid Medium, Fungi (12), with 10^3 arthroconidia per ml as previously described (4, 8). SCH 56592 was solubilized in dimethyl sulfoxide, and serial dilutions were then made in medium. Amphotericin B and itraconazole susceptibility testing was performed as described previously (6, 7). Depending on the growth rate of the isolate, MICs were

* Corresponding author. Mailing address: Division of Infectious Diseases, Santa Clara Valley Medical Center, 751 South Bascom Ave., San Jose, CA 95128-2699. Phone: (408) 998-4557. Fax: (408) 998-2723. E-mail: kv.clemons@juno.com.

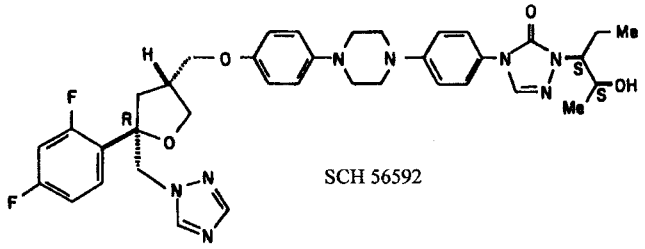

FIG. 1. Structural formula of SCH 56592.

determined after 6 to 7 days of static incubation at ambient temperature. The minimum fungicidal concentration (MFC), defined as >96% killing of the original inoculum, was determined by placing 0.05-ml samples from tubes with no growth onto 2% glucose–1% yeast extract agar and examining the plates for colonies after 3 to 5 days of incubation at ambient temperature.

Infection model. A model of systemic coccidioidomycosis was established in 6-week-old female CD-1 mice (mean weight, 24 g) by intravenous injection of 220 viable arthroconidia of the Silveira strain of *C. immitis* as described previously (3–8). SCH 56592 was solubilized in a solution of methylcellulose (M-0512; Sigma, St. Louis, Mo.)–Tween 80–NaCl. Four grams of methylcellulose in 1 liter of distilled water was heated with stirring at 80°C for 1.5 h followed by the addition of 5.6 ml of Tween 80 and 9 g of NaCl. The solution was autoclaved prior to the addition of SCH 56592. The doses of SCH 56592 used were 0.5, 2, 10, or 25 mg/kg of body weight per day. Fluconazole (Pfizer, Groton, Conn.) was solubilized in 0.3% noble agar in distilled water. It was given at either 10 or 100 mg/kg/day. Itraconazole (Janssen Pharmaceutica, Beerse, Belgium) was solubilized in 2-hydroxypropyl-β-cyclodextrin (American Maize Co., Hammond, Ind.) as described previously (13) and given at either 10 or 100 mg/kg/day.

Therapy was begun 2 days postinfection. Ten groups of 10 or 11 mice received either SCH 56592, fluconazole, itraconazole, methylcellulose diluent, or no treatment. All mice were treated by gavage once daily with the dose given in a volume of 0.1 ml. The mice were housed five or six per cage. Sterilized food and acidified water were provided ad libitum. Therapy was continued for 19 days, and deaths were recorded through 49 days postinfection. All surviving mice were euthanized by CO_2 asphyxiation. Quantitation of residual burdens of *C. immitis* in the spleen, liver, and lungs was done as described previously (4–8). In brief, organs were removed aseptically and homogenized in 5 ml of sterile saline containing antibiotics. Serial dilutions of the organ homogenates were placed onto Mycosel agar and incubated at 37°C to determine the number of viable CFU in each organ.

Statistics. Statistical analyses of survival and comparative burdens of *C. immitis* in the organs were done as described previously by either a Wilcoxon rank sum or a Mann-Whitney U test (4, 5, 7).

RESULTS

In vitro activity. The results of the in vitro data are given in Table 1. All five isolates of *C. immitis* tested were susceptible to low concentrations of SCH 56592, with the MICs ranging from 0.39 to 3.13 μg/ml. The MICs of SCH 56592 are similar to those of amphotericin B and itraconazole, which ranged from 1 to 2 and from 0.2 to 0.78 μg/ml, respectively (Table 1). SCH 56592 also showed good fungicidal activity, with MFCs for all five isolates being four- to eightfold higher than the MIC. In contrast, one of the five isolates showed resistance to amphotericin B, with an MFC of >8.0 μg/ml, and another isolate showed resistance to itraconazole with an MFC of >12.5 μg/ml (Table 1).

TABLE 1. Comparative in vitro susceptibilities of isolates of *C. immitis* to SCH 56592, amphotericin B, and itraconazole[a]

Isolate	SCH 56592		Amphotericin B		Itraconazole	
	MIC	MFC	MIC	MFC	MIC	MFC
Silveira	0.39	3.13	1.0	4.0	0.78	1.56
94-178	0.39	1.56	2.0	2.0	0.39	3.13
94-208	0.39	1.56	1.0	>8.0	0.20	3.13
93-110	3.13	3.13	1.0	1.0	0.39	0.39
93-154	1.56	3.13	2.0	2.0	0.78	>12.5

[a] All concentration values are in micrograms per milliliter.

In vivo activity. The survival data from the murine model of systemic coccidioidomycosis are shown in graphic form in Fig. 2. The two control groups had substantial mortality (90 to 100%) clustered over a period of several days commencing on day 12 postinfection through day 22. In contrast, 70 to 100% of treated animals survived through the course of the experiment. All three drugs at the various doses tested prolonged survival significantly compared with groups given no treatment or diluent-treated control animals ($P < 0.001$); all drug regimens were equivalent in the prolongation of survival. The lowest dose of SCH 56592 studied (0.5 mg/kg) produced survival equivalent to that with fluconazole or itraconazole administered at 10 mg/kg. Thus, it can be inferred that SCH 56592 is about 20-fold more active on a milligrams-per-kilogram basis in prolonging survival than either fluconazole or itraconazole.

The relative efficacies of the drugs in terms of reduction of mean CFU per individual organ were examined also and are presented in Table 2. In the spleen, the efficacies of itraconazole at 10 mg/kg and fluconazole given at 10 mg/kg were equivalent ($P > 0.05$), but both were inferior to itraconazole or fluconazole given at 100 mg/kg ($P < 0.01$ and 0.05 to 0.01, respectively). Fluconazole at 100 mg/kg was inferior to itraconazole at 100 mg/kg ($P < 0.01$). Itraconazole at 100 mg/kg achieved an approximately 95-fold greater reduction in the number of CFU per spleen than did the equivalent dose of fluconazole. SCH 56592 given at 0.5 mg/kg reduced the number of CFU recovered from the spleen comparably to itraconazole at 100 mg/kg ($P > 0.05$) and was significantly more active than 100 mg of fluconazole per kg ($P < 0.001$). SCH 56592 at 10 or 25 mg/kg was equivalent and superior to all other drug regimens ($P < 0.05$ to 0.001, depending on comparison). Thus, in the spleen, SCH 56592 was approximately 200-fold more potent on a milligrams-per-kilogram basis compared with itraconazole and was almost 20,000-fold as potent on a weight basis compared with fluconazole.

In the liver, there was no significant difference ($P > 0.05$) among fluconazole at 10 mg/kg, itraconazole at 10 mg/kg, and fluconazole at 100 mg/kg. SCH 56592 at 2 mg/kg was not significantly different from itraconazole at 100 mg/kg ($P > 0.05$). Thus, SCH 56592 was approximately 50-fold more potent than itraconazole in reduction of the number of CFU recovered from the liver. SCH 56592 at 0.5 mg/kg was superior to fluconazole at 100 mg/kg ($P < 0.01$), achieving an almost fourfold greater reduction in the number of CFU. Thus, SCH 56592 was about 200-fold more potent than fluconazole in reduction of the number of CFU recovered from the liver.

In the lungs, fluconazole at 10 or 100 mg/kg and itraconazole at 10 mg/kg were not significantly different. Itraconazole at 100 mg/kg was superior to those three regimens ($P < 0.01$, 0.01, and 0.001, respectively). The efficacy of SCH 56592 at 0.5 mg/kg was comparable with that of itraconazole at 100 mg/kg and was thus approximately 200-fold as potent. Compared with fluconazole, SCH 56592 was about 20,000-fold as potent.

There was a clear dose-response relationship for SCH 56592 at 0.5 to 10 mg/kg in all three organs examined. However, there was no further significant reduction in the mean number of CFU when the dose was escalated from 10 to 25 mg/kg.

When the numbers of individual organs cleared of infection with escalation of the dose of SCH 56592 were examined, it was apparent that the infection in the spleen was eliminated most readily, followed by that in the lungs, with the liver being the organ most difficult from which to eradicate the infection. No dose of either fluconazole or itraconazole cured any mice, i.e., no mouse was free of detectable *C. immitis* in all three

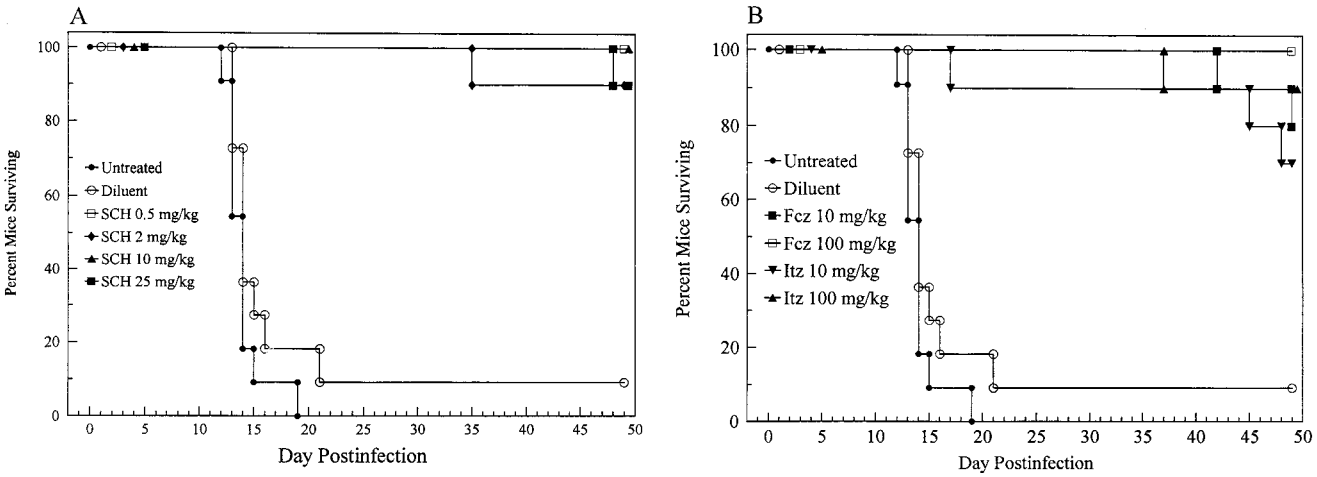

FIG. 2. Cumulative mortality of mice infected with *C. immitis* that had been given one of the various treatment regimens. (A) SCH 56592, diluent, or no treatment. (B) Itraconazole, fluconazole, diluent, or no treatment. Abbreviations: Diluent, methylcellulose diluent used for SCH 56592; SCH, SCH 56592; Fcz, fluconazole; Itz, itraconazole.

organs tested. In contrast, SCH 56592 administered at doses of 10 and 25 mg/kg cured 60 and 90% of the mice, respectively.

DISCUSSION

In terms of its ability to cure mice infected with *C. immitis*, SCH 56592 was comparable with SCH 51048, previously studied in this laboratory (8). SCH 39304, when administered at a dose of 50 mg/kg, was also able to cure the majority of animals experimentally infected with *C. immitis* (4). Unfortunately, further development of SCH 39304 was stopped due to carcinogenicity in rodents (14). Another experimental triazole, DO870 (Zeneca Pharmaceuticals, Macclesfield, Cheshire, England), in a previous series of experiments was able to cure only 20 to 30% of mice infected systemically with *C. immitis* (7). In other experiments with murine coccidioidomycosis, both lipid-complexed and conventional amphotericin B were able to effect varying percentages of cures in the systemically infected animals (5, 6), but amphotericin B of course must be administered parenterally.

In conclusion, SCH 56592 on a milligrams-per-kilogram of body weight basis was superior in activity to both fluconazole and itraconazole in prolonging the survival of mice given an otherwise lethal inoculum of *C. immitis*. SCH 56592 is at least 50- to 200-fold more potent on a weight basis in reducing the number of CFU in organs of surviving animals compared with itraconazole and fluconazole, respectively. In striking contrast to fluconazole and itraconazole, the two highest doses of SCH 56592 tested effected cures in the majority of the treated animals and thus can be considered possibly fungicidal in vivo. SCH 56592 should be given serious consideration for trials with patients infected with coccidioidomycosis, particularly those not responding to or those relapsing after treatment with currently available agents.

TABLE 2. Recovery of *C. immitis* from the organs of surviving mice treated with SCH 56592, fluconazole, or itraconazole

Group and dose (mg/kg)	No. of survivors/no. of survivors cured	Geometric mean $\log_{10}$ CFU in survivors (no. free of infection)		
		Spleen	Liver	Lung
Untreated[a]	0/0			
Methylcellulose[a]	1/0	3.79	3.54	5.46
Fluconazole				
10	8/0	3.89 (0)	3.95 (0)	4.84 (0)
100	10/0	3.68 (0)	4.22 (0)	4.80 (0)
Itraconazole				
10	7/0	4.22 (0)	4.12 (0)	4.94 (0)
100	9/0	1.11 (3)	1.61 (2)	2.41 (1)
SCH 56592				
0.5	10/0	1.99 (1)	3.63 (0)	3.03 (0)
2	9/1	0.61 (6)	1.91 (2)	1.89 (4)
10	10/6	0.25 (9)	0.76 (6)	0.59 (8)
25	9/9	0.0 (9)	0.0 (9)	0.0 (9)

[a] Each control group had 11 mice. All other treatment groups contained 10 mice.

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